

Role of Vitamin Intake and Risk of Cardiovascular Disease in
Overweight/Obese Dysglycemic African Americans and White
Americans

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I. Abstract

An increase in Vitamin C, E, and Carotenoids have been associated with lower risks of cardiovascular disease because of their ability to act as antioxidants. These vitamins work to inhibit the oxidation of low-density lipoprotein (LDL) cholesterol, which plays an important role in plaque formation in major blood vessels. In the current study, we compared the dietary intake of Vitamins E, C, and Carotenoid with lipids, lipoprotein, insulin sensitivity, body weight, and inflammation in overweight/obese African Americans (AA) and Whites Americans (WA) with dysglycemia. The purpose of this study was to examine the relationship between these dietary vitamins and the risk of cardiovascular disease in overweight/obese dysglycemic subjects, as well as examine these differences with regards to the race of the subjects. In our study, subjects were 24 WA (Mean age= 43 ± 14 years, mean BMI 37 ± 5 kg/m², mean BP 129/79) and 25 AA (Mean age= 45 ± 9 years, mean BMI 38 ± 6 kg/m², mean BP 127/81). All subjects were placed on a diet-induced weight loss program (Slimfast 3-2-1) for 6 months, and attended a program that included nutrition and lifestyle changes education. Subjects completed a food frequency questionnaire (FFQ) (Viocare Inc.), which compiled their nutrient intake over the past 3 months. Standard oral glucose tolerance tests (fasting and 2-hour glucose and insulin) were completed by all subjects. In addition, fasting blood levels for cholesterol, HDL-C, LDL-C, triglycerides, Apo A-1, ApoB, CRP, and oxidized LDL were obtained. Insulin Resistance was measured by homeostatic model assessment (HOMA-IR). Results indicated a significant increase in Vitamin E intake in WA and a significant increase in Vitamin E, C, and Carotenoids in AA over a 6 month period. We found no significant changes in HDL-C, LDL-C, oxidized LDL, or CRP in our groups. There were significant differences in systolic blood pressure, pulse, and waist circumference between AA and WA. Based on the RDI, the mean intake of Vitamin C, E, and Carotenoids were within the normal range of the general population's intake at the beginning of the study, and above the normal range after 6 months. Further studies are warranted to examine this relationship between dietary intake of antioxidants and their ability to lower cardiovascular disease risk.

II. Introduction

It is well known that cardiovascular disease is the leading cause of death in the United States, with 1 in 3 Americans having the disease, and accounting for 36.3% of deaths in America—accounting for more deaths than any other cause [1]. While some of the risks for developing cardiovascular disease can be attributed to genetic factors, many are influenced by dietary or lifestyle factors, and are therefore able to be modified to reduce cardiovascular disease risk. Among these factors that can be modified are diet, physical activity, and body weight. These factors can also modify high blood pressure, diabetes mellitus, high blood cholesterol, and obesity, all of which are associated with cardiovascular disease. In the general population, overweight is defined as a BMI of 25.0 to 29.9 kg/m², and obesity is defined as a BMI ≥ 30.0 kg/m² [2]. According to an National Health and Nutrition Examination Survey (NHANES) study, 65% of U.S. adults are either overweight or obese. Obesity is associated with other cardiovascular disease risk factors including increased hypertension, glucose intolerance, and increased inflammation. Recent studies have also shown an increased correlation between obesity and metabolic syndrome [2]. Metabolic syndrome is a combination of physical and physiological abnormalities such as large waist circumference, high triglyceride levels, and low high-density lipoprotein (HDL) levels [3]. Metabolic Syndrome has been linked with increased oxidative stress and inflammatory markers, such as CRP and oxidized LDL, typically associated with cardiovascular disease [4]. A NHANES study looking at metabolic syndrome and antioxidants found that lower concentrations of Vitamin C and Carotenoids were associated with an increase in metabolic syndrome criteria when compared to subjects that did not have any of the criteria for metabolic syndrome. [5]. Higher intake of fruits and vegetables are also correlated with a lower risk of metabolic syndrome [6].

Cardiovascular disease involves the process of atherosclerosis, which is the buildup of plaque on the walls of major arteries. On a microscopic level, a major factor in the development of atherosclerosis is the oxidation of low-density lipoprotein (LDL) cholesterol. Once the LDL cholesterol becomes oxidized, it moves

to the endothelial lining of a major artery, and begins to start accumulating many inflammatory cells to the artery. The accumulation of these inflammatory cells to the damaged artery forms fatty streaks that lead to atherosclerotic plaque that can start to restrict blood flow and eventually cause a heart attack. Reactive oxygen species are partly responsible for this LDL oxidation. These oxidation events may be inhibited by antioxidants. These antioxidants can be introduced into the diet by increasing fruit and vegetable intake [7] or through the supplementation of vitamins that act as antioxidants, such as vitamin A, vitamin C, and Carotenoids.

Higher intake of vitamins E, C, and Carotenoids are typically associated with decreased risk of cardiovascular disease [8]. These vitamins work as antioxidants through their mechanism of scavenging free radicals and other oxidative species before they can initiate oxidation of LDL cholesterol. Each of these vitamins have a different mechanism that achieve the same function of preventing this oxidation. Vitamin E terminates free radicals by scavenging peroxy radicals [9]. This inhibits the propagation of free radicals in plasma lipoproteins and membranes [10]. Vitamin E also works with Vitamin C to maintain a proper antioxidant and free radical balance [10]. Vitamin C acts as a reactive oxygen species scavenger by donating an electron to the species before it can oxidize another molecule [12]. Carotenoids such as lycopene and xanthophyll also work to reduce the reactive oxygen species, thus protecting the LDL cholesterol from oxidation. [13]. It is because of these functions that the American Heart Association Science Advisory committee recommended a diet containing antioxidant-rich whole fruits and vegetables to help prevent cardiovascular disease [14].

While in theory, the ability of these antioxidant vitamins to protect LDL cholesterol from oxidation, results from recent studies have demonstrated unclear and varying results. A 2002 follow up study from the National Health and Nutrition Examination Survey found decreased cardiovascular disease mortality with an intake of 3 servings of fruits and vegetables per day. The study also stated that the roles of these vitamins may be enhanced by the other components present in whole foods [15]. An additional study confirmed the inverse correlation between antioxidant containing fruit and vegetable intake with the risk of cardiovascular

disease in healthy subjects. This study concluded that having various cardiovascular disease risk factors, such as diabetes or hypertension, could lead to a reduced intake of the antioxidants and mask associations between intake and cardiovascular disease risk [16]. While this study showed a positive effect of vitamins' role in protecting against cardiovascular disease, many other studies—especially those involving vitamin supplementation—have not shown significant correlations between these vitamins and cardiovascular disease [7,14,18]. A 2011 study involving Vitamin C concluded that Vitamin C supplementation was not associated with decreased cardiovascular disease or major cardiovascular events in subjects who were already at high risk for cardiovascular disease and who had already had heart attacks [17]. These vitamins in supplement form have also been shown to have limited effects on the oxidation of LDL cholesterol, but have had some effects subjects get their vitamins from whole foods rather than supplements [7,14,18]. This suggests that there are multiple factors associated with cardiovascular disease risk, such as absorption, and healthy versus at risk subjects. Therefore, further studies are warranted.

African Americans have been typically shown to have a higher risk of cardiovascular disease than the general population, despite having higher high-density lipoprotein cholesterol levels and lower triglyceride levels when compared to WA [19]. While differences in vitamin intake has not been found between AA and WA, various biomarkers for cardiovascular disease have shown differences in response to vitamin intake. For example, a decrease in Carotenoid intake were associated with an increase in insulin resistance in African Americans but not in whites [20]. Even though these findings indicate differences, the reason behind the increased risk still remains unclear.

Purpose

The purpose of this study was examine changes in anthropometric values, vitamin intake, lipid and lipoprotein levels, inflammatory markers, glucose metabolism, and insulin resistance in response to a 6 month diet-induced weight loss study among AA and WA. This study also examined ethnic differences among these groups.

III. Methods

III.1 Anthropometrics

Subjects general anthropometric and body composition measurements were taken over the 6 month period. The measurements included height (in cm with shoes off), weight (in kg), BMI (calculated from height and weight), waist circumference, blood pressure, and pulse. Their blood pressure was taken by an Omron Blood Pressure Monitor [24] while the subjects were sitting down, after resting for 10 minutes.

III.2 Food Recall

Subjects completed a food frequency questionnaire [23], which compiled their general nutrient intake over the past 3 months. The Viocare food frequency questionnaire (FFQ) involved selecting food from a general list (i.e. cold breakfast cereals or corn tortillas), and indicating how many times over the past 3 months those foods were eaten. The FFQ calculated their carbohydrate, protein, fat, and vitamin intake based on their answers. This was completed at baseline and 6 months of the study.

III.3 Metabolic Studies

Standard Oral Glucose Tolerance tests (glucose and insulin) were performed at the baseline and 6 month mark. Fasting and 2-hour glucose and insulin were drawn in all subjects. In our study, dysglycemia were evaluated from these results. Dysglycemia was defined as individuals who have a fasting serum glucose between 100 -125 mg/dL. The Insulin Resistance was calculated using the Homeostasis Model Assessment (HOMA-IR) at the baseline and 6 month marks [24]. The HOMA-IR model uses the fasting plasma insulin and glucose concentrations to estimate insulin resistance. The equation used to calculate the HOMA-IR was:

$$HOMA-IR = (FPI \times FPG) / 22.5 [24]$$

In this equation FPI is fasting plasma insulin and FPG is fasting plasma glucose.

Lipids, Lipoproteins, and Inflammatory Markers

In addition, fasting blood levels for cholesterol, high-density lipoproteins (HDL)-C, low-density lipoproteins (LDL-C), triglycerides, apo lipoprotein A (Apo A-

1), apo lipoprotein B (ApoB), c-reactive protein (CRP), and oxidized LDL was measured using oxidized low-density lipoprotein (Oxd LDL) in all subjects.

Statistical analysis was performed to examine the differences at the baseline and 6-month mark. We used mean and standard deviation to report changes. We also examined racial differences in these results.

III.4 Weight Loss Intervention and Education Program

All subjects were placed on a diet induced weight loss program for 6 months. This dietary program consisted of meal replacements with Slim-Fast 3-2-1 [20]. This low calorie diet contained 3 snacks (100 calories each), 2 meal replacement shakes, and 1 balanced meal. The additional meal consisted of 50% carbohydrates, 30% fat, and 20% protein, and the Slim-Fast had 180-190 kcal each (9 g total fat; 4 g total carbohydrate, 20 g protein, and 2 g fiber). These Slim-Fast shakes were also supplemented with 100% Vitamin E, 100% Vitamin C, and 35% Carotenoids. Subjects also attended the Comprehensive Weight Loss Management Program (CWP) at the Ohio State University. This was a 6 month lifestyle and education program where participants met weekly. Topics discussed were good nutrition, healthy weight loss tips, and physical activity[22]. Subjects were given selections of healthy snacks, as well as consultation of how to put together healthy meals. Subjects were weighed in bi-weekly, and consulted on their dietary changes.

IV. Results

IV.1 Anthropometric Results

The subjects in our study were 24 White Americans (mean age 43 ± 14 years; mean BMI 37 ± 5 kg/m²; mean BP 127/81 mmHg) and 25 African Americans (mean age 45 ± 9 years, mean BMI 38 ± 6 kg/m²; mean BP 127/81 mmHg).

The anthropometric data for the subjects can be found in **Table 1**. While the White American subjects exhibited both significant weight loss (-10.9754 ± 1.33 kg; $P < 0.0001$) and a decrease in BMI (-5.1760 ± 1.17 kg/m²; $P < 0.0001$), African American subjects exhibited a significant decrease in weight loss (-5.0400 ± 1.30 kg;

P=0.0003), but no significant decrease in BMI (-1.8568 ± 1.15 kg/m²; P=1.1123). The waist circumference of the White American subjects significantly decreased -9.9742 ± 1.62 cm (P<0.0001), and the waist circumference of the African American subjects had a decrease -3.3232 ± 1.59 cm (P=0.0419), which was not significant. White Americans had a significant decrease in systolic and diastolic blood pressure (-10.5833 ± 2.04 mmHg (P<0.0001) and -6.4583 ± 1.46 mmHg (P<0.0001) respectively). African Americans had an insignificant decrease in systolic and diastolic blood pressure (-3.3200 ± 2.00 mmHg (P=0.1030) and -2.8400 ± 1.43 mmHg (P=0.0537) respectively).

We found racial differences in our groups in response to weight loss with regards to weight, waist circumference. These values only different at the 6 month mark, not at the baseline. White Americans lost 5.94 ± 1.86 kg (P=0.0025) and 6.651 ± 2.27 cm (P=0.0052) of waist circumference more than African American subjects. In addition, we found differences in systolic blood pressure and pulse. White Americans decreased their systolic blood pressure and average pulse 7.26 ± 2.85 mmHg (P=0.0142) and 7.32 ± 2.71 beats/min (P=0.0095) more than African American subjects.

Table 1A and 1B: Anthropometric results at baseline and 6 months of a diet-induced weight loss program.

Table 1A	Baseline-White Americans	6 Months-White Americans	Mean Difference	Standard Deviation	P-Value
Weight (kg)	106.18	95.2	-10.9754	1.33	<0.0001*
Height (cm)	169.92	234.21	64.2938	45.02	0.1598
BMI (kg/m ²)	36.53	31.36	-5.176	1.17	<0.0001*
Systolic BP (mmHg)	129.08	118.5	-10.5833	2.04	<0.0001*
Diastolic BP (mmHg)	79.42	72.96	-6.4583	1.46	<0.0001*
Pulse (Beats/Min)	72.58	65.71	-6.875	1.93	0.0009*
Waist Circumference (cm)	116.51	106.54	-9.9742	1.62	<0.0001*
Table 1B	Baseline-African Americans	6-Months-African Americans	Mean Difference	Standard Deviation	P-Value
Weight (kg)	105.72	100.68	-5.04	1.3	0.0003*
Height (cm)	166.11	166.11	0	44.11	1
BMI (kg/m ²)	38.33	36.47	-1.8568	1.15	0.1123
Systolic BP (mmHg)	127.2	123.88	-3.32	2	0.103
Diastolic BP (mmHg)	80.92	78.08	-2.84	1.43	0.0537
Pulse (Beats/Min)	72.08	72.52	0.44	1.9	0.8173
Waist Circumference (cm)	114.51	111.19	-3.3232	1.59	0.0419

BMI calculated from $\frac{\text{Weight (kg)}}{\text{Height (m)}^2}$ equation; BMI, body mass index
BP, Blood Pressure

* Indicates significant changes over the 6-month period.

Difference is significant if the P-value is below 0.0161

IV.2 Nutrient Results

The nutrient changes in the subjects can be viewed in **Table 2**. While both subjects did decrease their caloric intake, only White American subjects had a significant decrease of -798.25 ± 231.77 kcal ($P=0.0012$) over the 6 month period; African American subjects decreased their caloric intake -412.40 ± 227.08 kcal ($P=0.0757$). White Americans changed their carbohydrate intake by -61.25 ± 28.68 g ($P=0.0380$) and $+6.50 \pm 1.67\%$ ($P=0.0003$) over the 6-month period. Their fat intake decreased -51.25 ± 12.31 g ($P=0.0001$) and $-9.08 \pm 1.60\%$ ($P<0.0001$). They decreased their saturated fats, monounsaturated fats, and polyunsaturated fats -19.09 ± 4.62 g ($P=0.0001$), -17.36 ± 4.70 g ($P=0.0006$), and -9.42 ± 2.37 g ($P=0.0002$) respectively. Their omega-3 intake increased 0.0417 ± 0.03 g ($P=0.2359$). Their grams of protein decreased -19.29 ± 9.05 ($P=0.0383$), but their protein percentage increased $2.92 \pm 0.73\%$ ($P=0.0002$). Their cholesterol intake decreased -175.17 ± 37.54 g ($P<0.0001$).

Their intake of Vitamin E, C, and Carotenoids increased 14.23 ± 3.47 mg ($P=0.0002$), 39.46 ± 20.96 mg ($P=0.0660$), and 236.56 ± 229.42 μ g ($P=0.3078$) respectively after 6 months. Only the vitamin E intake in our WA subjects had a significant change.

In our African American subjects their carbohydrate intake changed -5.40 ± 28.10 g ($P=0.8484$) and $+6.200 \pm 1.63\%$ ($P=0.0004$) over the 6-month period. Their fat intake decreased -36.56 ± 12.06 g ($P<0.0001$) and $-96.96 \pm 1.57\%$ ($P=0.0039$). They decreased their saturated fats, monounsaturated fats, and polyunsaturated fats -13.0440 ± 4.53 g ($P=0.0060$), -13.02 ± 4.60 g ($P=0.0069$), and -7.86 ± 2.32 g ($P=0.0014$) respectively. Their omega-3 intake decreased 0.04 ± 0.03 g ($P=0.2453$). Their grams of protein decreased -9.40 ± 8.87 ($P=0.2946$), but their protein percentage increased $1.28 \pm 0.72\%$ ($P=0.0804$). Their cholesterol intake decreased -115.88 ± 36.78 g ($P=0.0804$).

Their vitamin intake of Vitamin E, C, and Carotenoids all increased significantly, seeing changes of 14.04 ± 3.40 mg ($P=0.0001$), 82.04 ± 20.54 mg ($P=0.0002$), and 715.22 ± 224.79 μ g ($P=0.0026$) respectively.

At both baseline and 6 months, the vitamin intake between the WA and AA subjects were not significantly different. The specific changes of vitamin intake before and after the weight loss program can be seen in **Figures 1 and 2**.

Table 2 A and B: Nutrient Intake at baseline and 6 months of a diet induced weight loss program.

Table 2A		Baseline-White Americans	6 Months-White Americans	Mean Difference	Standard Deviation	P-Value
	Caloric Intake (kcal)	2759.13	1960.88	-798.25	231.77	0.0012*
	Carbohydrates (g)	310.75	249.5	-61.25	28.68	0.038
	Carbohydrates (% Total Diet)	43.83	50.33	6.5	1.67	0.0003*
	Fats (g)	116.67	65.42	-51.25	12.31	0.0001*
	Fats (% Total Diet)	37.46	28.38	-9.0833	1.6	<0.0001*
	Saturated Fats (g)	41.21	22.12	-19.0875	4.62	0.0001*
	Monounsaturated Fats (g)	42.68	25.32	-17.3625	4.7	0.0006*
	Polyunsaturated (g)	22.27	12.85	-9.4167	2.37	0.0002*
	Omega-3 (g)	0.11	0.15	0.0417	0.03	0.2359
	Protein (g)	120.67	101.38	-19.2917	9.05	0.0383
	Protein (%)	17.54	20.46	2.9167	0.73	0.0002*
	Cholesterol (g)	412.21	237.04	-175.17	37.54	<0.0001*
	Vitamin E (mg)	17.66	31.89	14.225	3.47	0.0002*
	Vitamin C (mg)	114.5	153.96	39.4583	20.96	0.066
	Carotenoids (µg)	792.89	1029.45	236.56	229.42	0.3078
Table 2B		Baseline-African Americans	6-Months-African Americans	Mean Difference	Standard Deviation	P-Value
	Caloric Intake (kcal)	2330.48	1918.08	-412.4	227.08	0.0757
	Carbohydrates (g)	266.12	260.72	-5.4	28.1	0.8484
	Carbohydrates (% Total Diet)	45.68	51.88	6.2	1.63	0.0004*
	Fats (g)	103.32	66.76	-36.56	12.06	<0.0001*
	Fats (% Total Diet)	37.88	30.92	-6.96	1.57	0.0039*
	Saturated Fats (g)	35.9	22.86	-13.044	4.53	0.0060*
	Monounsaturated Fats (g)	37.97	24.95	-13.02	4.6	0.0069*
	Polyunsaturated (g)	21.59	13.73	-7.86	2.32	0.0014*
	Omega-3 (g)	0.2	0.16	-0.04	0.03	0.2453
	Protein (g)	90.72	81.32	-9.4	8.87	0.2946
	Protein (%)	15.56	16.84	1.28	0.72	0.0804
	Cholesterol (g)	338.6	222.72	-115.88	36.78	0.0028*
	Vitamin E (mg)	17.47	31.51	14.044	3.4	0.0001*
	Vitamin C (mg)	85.2	167.24	82.04	20.54	0.0002*
	Carotenoids (µg)	824.31	1539.53	715.22	224.79	0.0026*

Kcal, kilocalories

% Total Diet is the percent of each macronutrient represented in the total calorie intake. Carbohydrates and Protein both yield 4 kcal/g, Fat yields 9 kcal/g.

*Indicates significant changes over a 6 month period.

The mean difference is significant if the P-value is less than 0.0161

Figures 1 and 2: Vitamin Intake in Subjects at baseline and 6 months of a diet induced weight loss program compared to National Average and RDI.

Figure 1

Vitamin Intake in White Americans over a 6-Month Period on a Diet Induced Weight Loss Program compared to National Average and RDI

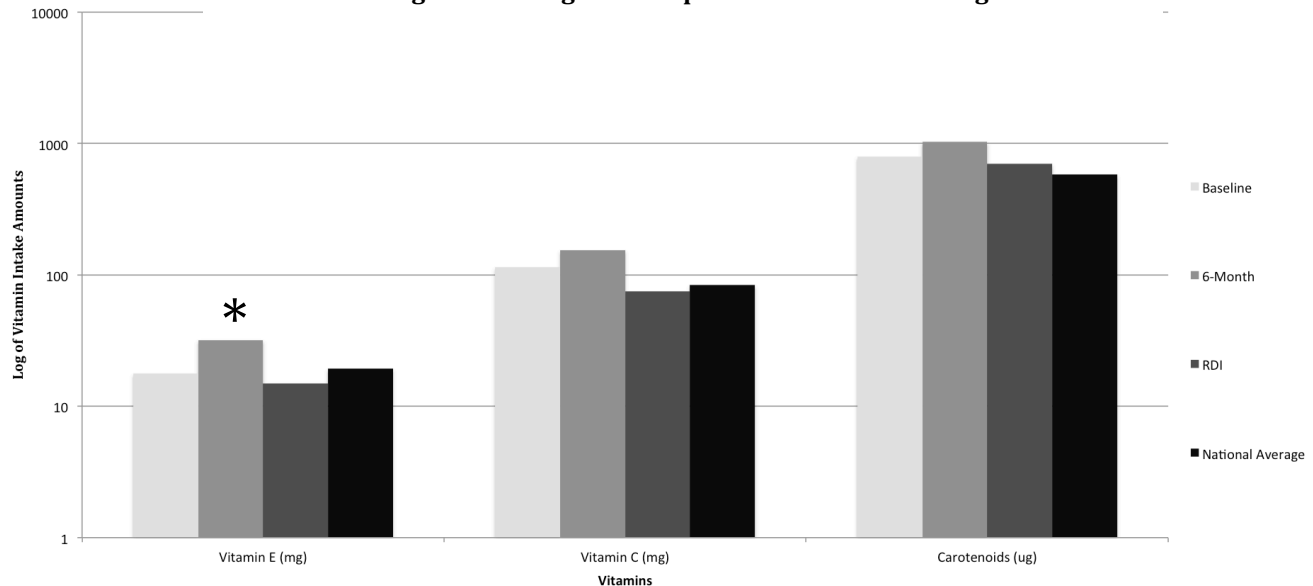
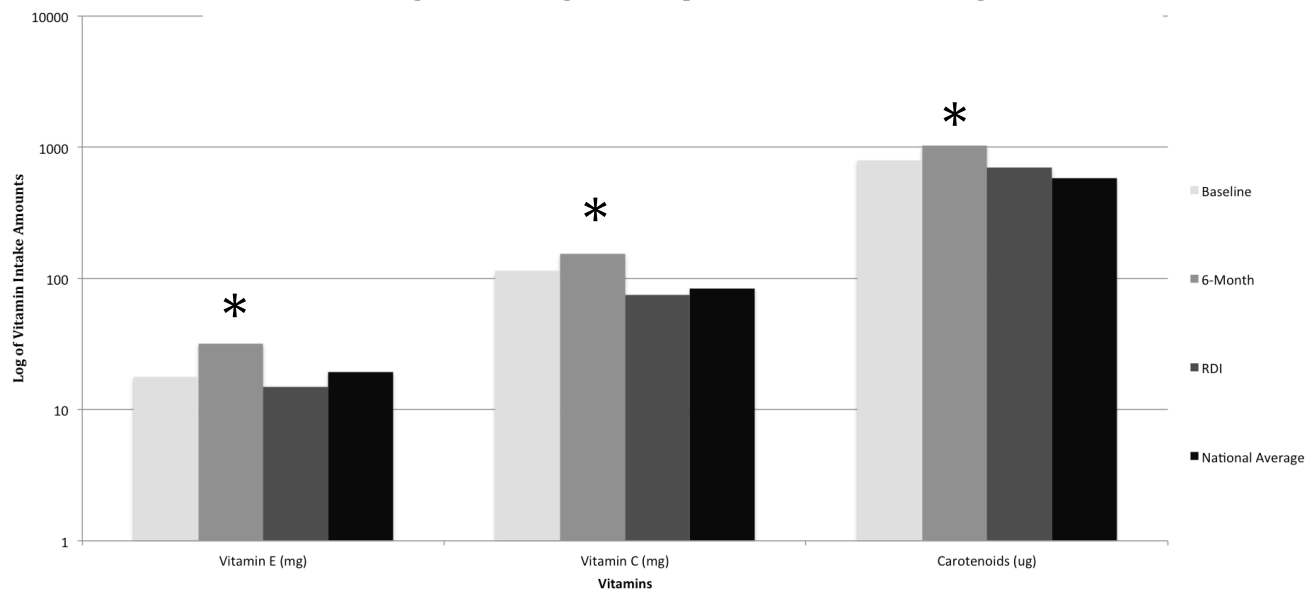


Figure 2

Vitamin Intake in White Americans over a 6-Month Period on a Diet Induced Weight Loss Program compared to National Average and RDI



The RDI, Recommended Daily Intake[26]. RDI For Vitamin E= 15 mg/day; Vitamin C 75-90 mg/day; Carotenoids= 700-900 µg/day.

National Average [26]

*Indicates significant change over the 6 month period

The mean difference is significant if the P-value is less than 0.0161

IV.3 Metabolic Studies

The serum glucose and hemoglobin A1C levels are shown in **Table 3 A & B**. Insulin resistance was measured through HOMA-IR [25]. White American subjects had a decrease in fasting blood glucose of -2.29 ± 2.86 mg/dL ($P=0.4270$), and a decrease in 2-hour blood glucose of -13.92 ± 6.91 mg/dL ($P=0.0499$) over the 6 month period. The hemoglobin A1C levels decreased by $-0.20 \pm 0.07\%$ ($P=0.0064$) over the 6-month period. The HOMA-IR in the White American subjects decreased by -0.72 ± 0.32 ($P=0.0297$). The African American subjects had a decrease of -4.08 ± 2.80 mg/dL ($P=0.152$) in their fasting blood glucose, and a decrease of -0.88 ± 6.78 mg/dL ($P=0.8972$) in their 2-hour blood glucose over the 6 month period. Their hemoglobin A1C decreased by $-0.15 \pm 0.07\%$ ($P=0.0318$). Their HOMA-IR decreased by -0.74 ± 0.31 ($P=0.0221$). The WA subjects had a decrease in fasting serum insulin of -2.59 ± 21.99 mg/dL ($P=0.0392$), and had a significant decrease in their 2-hr serum insulin of -38.20 ± 11.34 mg/dL ($P=0.0015$). The AA subjects saw a decrease in their fasting serum insulin of -2.90 ± 1.22 mg/dL ($P=0.0217$), and a decrease in their 2-hr serum insulin of -5.98 ± 11.11 mg/dL ($P=0.5926$). We found significant changes in A1C and 2-hr Insulin among WA subjects, but not AA subjects.

Table 3 A and B: Blood glucose and metabolic markers in subjects at baseline and 6 months of a diet-induced weight loss program.

Table 3 A	Baseline-White Americans	6 Months-White Americans	Mean Difference	Standard Deviation	P-Value
Total Cholesterol (mg/dL)	190.42	179.42	-11.0000	4.64	0.0221
HDL-C (mg/dL)	46.17	48.86	2.6957	1.50	0.0783
LDL-C (mg/dL)	117.08	108.30	-8.7826	4.08	0.0364
Triglycerides (mg/dL)	136.38	111.21	-25.1739	7.90	0.0026*
Apo A-1 (U/L)	139.88	141.97	2.0870	3.42	0.5453
Apo B (U/L)	96.88	88.57	-8.3043	2.61	0.0026*

Table 3 B	Baseline-African Americans	6-Months-African Americans	Mean Difference	Standard Deviation	P-Value
Total Cholesterol (mg/dL)	192.72	184.72	-8.0000	4.45	0.0790
HDL-C (mg/dL)	49.80	49.64	-0.1600	1.44	0.9117
LDL-C (mg/dL)	124.60	117.84	-6.7600	3.91	0.0905
Triglycerides (mg/dL)	91.92	82.04	-9.8800	7.57	0.1986
Apo A-1 (U/L)	148.00	142.76	-5.2400	3.29	0.1175
Apo B (U/L)	94.84	94.17	-0.6667	2.55	0.7952

2 hour blood glucose tolerance test was completed using 75 mg of glucose.

HOMA-IR, Homeostatic model assessment

A1C, Hemoglobin A1C

*indicates significant changes over the 6 month period.

The mean difference is significant if the P-value is less than 0.0161

IV.4 Lipids and Lipoproteins

The serum lipids and lipoprotein amounts can be seen in **Table 4 A & B**. The White American subjects decreased their total cholesterol by -11.00 ± 4.64 mg/dL ($P=0.0221$) over the 6 month period. Their HDL -C increased $+2.70 \pm 1.50$ mg/dL ($P=0.0783$), and their LDL-C decreased -8.78 ± 4.08 mg/dL ($P=0.0364$). Their triglycerides significantly decreased by -25.17 ± 7.90 mg/dL ($P=0.0026$). Apo lipoprotein A-1 and Apo lipoprotein B increased by 2.08 ± 3.42 U/dL ($P=0.5453$) and decreased by -8.30 ± 2.61 U/dL ($P=0.0026$) respectively. The African American subjects had a decrease of -8.00 ± 4.45 mg/dL ($P=0.0221$) in their total cholesterol over the 6-month period. Their HDL-C and LDL-C decreased by -0.16 ± 1.44 mg/dL ($P=0.9117$) and -6.76 ± 3.91 mg/dL ($P=0.0905$) respectively, but was not significant. Their triglycerides decreased -9.88 ± 7.57 mg/dL ($P=0.1986$). The Apo lipoprotein A1 and Apo lipoprotein B decreased -5.24 ± 3.29 U/dL ($P=0.1175$) and -0.67 ± 2.55 U/dL ($P=0.7952$). These can also be seen in **Figures 3 and 4**.

Table 4 A and B: Lipid and Lipoprotein levels in subjects at baseline and 6 months of a diet-induced weight loss program.

Table 4 A	Baseline-White Americans	6 Months-White Americans	Mean Difference	Standard Deviation	P-Value
Total Cholesterol (mg/dL)	190.42	179.42	-11.0000	4.64	0.0221
HDL-C (mg/dL)	46.17	48.86	2.6957	1.50	0.0783
LDL-C (mg/dL)	117.08	108.30	-8.7826	4.08	0.0364
Triglycerides (mg/dL)	136.38	111.21	-25.1739	7.90	0.0026*
Apo A1 (U/L)	139.88	141.97	2.0870	3.42	0.5453
Apo B (U/L)	96.88	88.57	-8.3043	2.61	0.0026*

Table 4 B	Baseline-African Americans	6-Months-African Americans	Mean Difference	Standard Deviation	P-Value
Total Cholesterol (mg/dL)	192.72	184.72	-8.0000	4.45	0.0790
HDL-C (mg/dL)	49.80	49.64	-0.1600	1.44	0.9117
LDL-C (mg/dL)	124.60	117.84	-6.7600	3.91	0.0905
Triglycerides (mg/dL)	91.92	82.04	-9.8800	7.57	0.1986
Apo A1 (U/L)	148.00	142.76	-5.2400	3.29	0.1175
Apo B (U/L)	94.84	94.17	-0.6667	2.55	0.7952

HDL-C, High Density Lipoprotein Cholesterol

LDL-C, Low Density Lipoprotein Cholesterol

ApoA1, Apoprotein A1

ApoB, Apoprotein B

* Indicates significant difference over the 6 month period

The mean difference is significant if the P-value is less than 0.0161

Figures 3 and 4: Cholesterol, Lipid, and Lipoprotein levels in subjects at baseline and 6 months of a diet-induced weight loss program.

Figure 3 Cholesterol, Lipid, and Lipoprotein Levels in White Americans on a Diet Induced Weight Loss Program

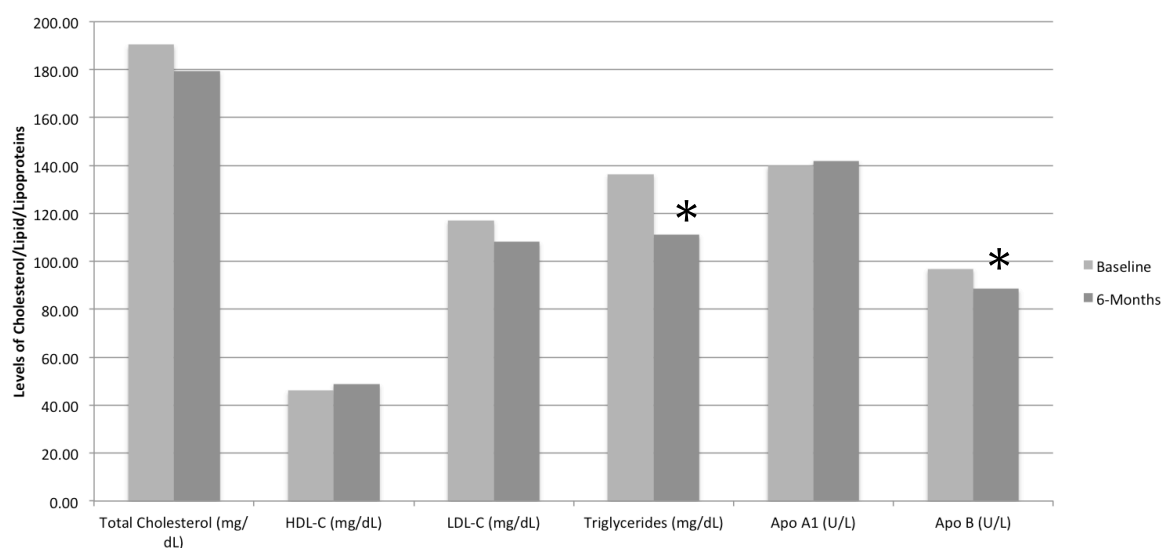
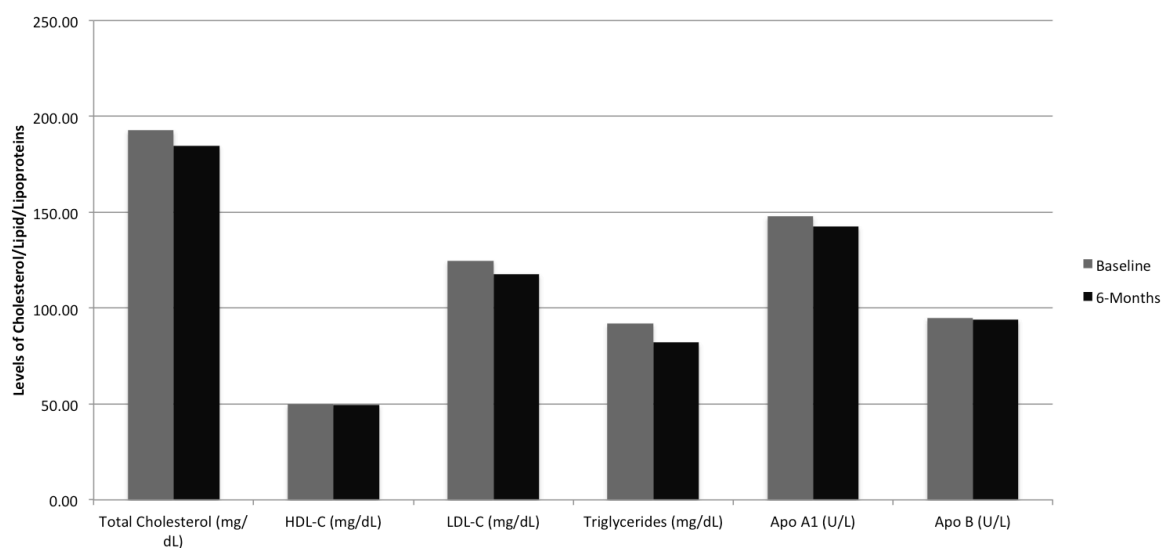


Figure 4 Cholesterol, Lipid, and Lipoprotein Levels in African Americans on a Diet Induced Weight Loss Program



*Indicates significant difference over the 6 month period
The mean difference is significant if the P-value is less than 0.0161

IV.5 Inflammation and Oxidized LDL

The changes of the inflammatory markers amounts can be viewed in **Table 5**. There were no significant changes in c-reactive protein or oxidized LDL levels over the 6 month period. The c-reactive protein (CRP) decreased -2.14 ± 1.01 mg/dL ($P=0.0400$) in the White American subjects, and the oxidized low-density lipoprotein increased 0.9043 ± 3.98 U/L ($P=0.8213$) over the 6-month period. The African American subjects had a decrease of -1.25 ± 0.95 mg/dL ($P=0.1932$) in their c-reactive protein, and an increase of 4.94 ± 3.52 U/L ($P=0.1679$) in their oxidized low-density lipoprotein over the 6 month period. None of these changes were statistically significant among our AA and WA subjects.

Table 5: Inflammatory marker levels in subjects at baseline and 6 months of a diet-induced weight loss program.

	Baseline-White Americans	6 Months-White Americans	Mean Difference	Standard Deviation	P-Value
CRP (mg/dL)	5.4183	3.2783	-2.14	1.01	0.04
Oxidized LDL (U/L)	51.5536	52.4579	0.9043	3.98	0.8213

	Baseline-African Americans	6-Months-African Americans	Mean Difference	Standard Deviation	P-Value
CRP (mg/dL)	8.022	6.7684	-1.2536	0.95	0.1932
Oxidized LDL (U/L)	47.6847	52.6272	4.9425	3.52	0.1679

CRP, c-reactive protein; Oxidized LDL, oxidized low-density lipoprotein

*Indicates significant difference over the 6 month period.

The mean difference is significant if the P-value is less than 0.0161

V. Discussion

V.1 Antropometric Results

This study demonstrated that the SlimFast 3-2-1 plan was successful in helping overweight and obese, dysglycemic subjects lose weight. The weight loss study was also successful in generally increasing the dietary intake of Vitamin E, C, and Carotenoid. A 1997 study [44] showed that over extended periods of weight loss, limiting certain foods, counting calories, and limiting quantities of food were successful at losing significant amounts of weight. Our study focused on replacing meals with meal replacement shakes and using professional help to make healthier choices, instead of the weight loss method of limiting certain foods. The study mentioned above occurred for a year, where as our study only occurred over a 6 month period. The lack of significant change in the BMI of African American subjects, the 6-month period did not yield enough weight loss to significantly lower the BMI of the African American subjects. We speculate that some of these weight loss changes could be attributed to the 100 calorie snacks and the meal that the individual subjects chose.

The weight loss program also did not significantly lower the waist circumference of the African American subjects over a 6-month period. The Look Ahead study [28], a weight loss intervention study incorporating decreased caloric intake and increased physical activity in subjects with type 2 diabetes, saw decreased waist circumference with decreased weight loss. The lack of significant waist circumference changes could indicate that more time is need to significantly decrease the circumference since the Look Ahead study took place over a year, rather than just 6 months. The Look Ahead study [29] also indicated that physical activity, not just decreased caloric intake alone, contributes greatly to decrease in BMI and waist circumference. The Diabetes Prevention Program [42,43] also included dietary, behavioral, and physical activity modifications in overweight and obese subjects. This study saw a decrease of weight in their subjects. This was also observed in the NHANES 1999-2002 survey where they observed an inverse relationship between physical fitness and BMI [30]. The above studies have similar lifestyle interventions that our subjects received counseling on during our study.

These studies indicate that the diet-induced weight loss program alone might not be as successful as a program with a combined diet and physical activity plan.

Our weight loss program had other important benefits. For example, we found that blood pressure significantly decreased in WA, but not in AA. Subjects were allowed to be on blood pressure medication during the study, and had a blood pressure below the high-risk blood pressure of 130/85 mmHg in patients with metabolic syndrome. [27] The significant decrease in blood pressure in the White American subjects indicates that the diet-induced weight loss program was successful of lowering blood pressure regardless of the medication. Subjects in the Diabetes Prevention Program and the Look Ahead Trial both reported decreases in blood pressure. [28,29, 42] This confirms the need for combined dietary and physical activity programs.

V.2 Metabolic Studies

In our study, dysglycemia was defined as having a fasting glucose and 2-hr blood glucose of 100-125 mg/dL and 140-199 mg/dL respectively, and A1C values between 5.7 and 7.0. Our subjects demonstrated significant decrease in fasting blood glucose from baseline to 6 months (WA subjects decreased from 102.17 mg/dL to 99.58 mg/dL ($P=0.427$); AA subjects decreased from 101.12 mg/dL to 97.04 mg/dL ($P=0.1520$)). This suggests that moderate weight loss is beneficial in decreasing dysglycemia. The A1C levels of the White American Subjects did significantly decrease by -0.2% ($P=0.0064$), indicating that the amount of glycated hemoglobin decreased over the 6-month period. The significant change was not observed in the African American subjects. The Look Ahead trial indicated significant changes in hemoglobin A1C amounts in subjects that combine dietary changes, physical activity, and behavioral modifications [28]. The Diabetes Prevention Program [42,43], which used a similar program including diet and behavior modifications in overweight and obese subjects, saw that the subjects reduced their risk of developing diabetes by 58%. We did not observe a significant change in the HOMA-IR value, indicating that the subjects insulin resistance did not significantly change in response to weight loss.

V.3 Caloric and Macronutrient Intake

The Slimfast 3-2-1 program helped subjects successfully lower their caloric intake, while also increasing their vitamin intake. The program was also successful in lowering the caloric intake of the subjects below the 2000 kcal that is recommended for the average American diet. In general, the WA caloric intake decreased from 2700 calories to 1960 calories over the 6 month period; and the AA caloric intake generally decreased from 2300 calories to 1900 calories over the 6 month period. While only the White American subjects differences were significant (-789.25 ± 231.77 ; $P=0.0012$), both groups were successful in lowering their caloric intake below the 2,000 calorie mark. The lack of significant changes in the African American subjects could be because they had a much lower caloric intake at the beginning of the study, therefore they did not have to reduce their caloric intake as much. The subjects' carbohydrate, fat, and protein ratios were also modified to be in the range of the recommended diet of 45-65% carbohydrates, 20-35% fats, and 10-35% protein. From this, we suspect that the subjects were successful in achieving a healthier proportion of macronutrients in their diet. The Center for Disease Control recommends having no more than 10% of our daily calories be from saturated fats in order to prevent cardiovascular disease [31]. Diets high in saturated fats and low in polyunsaturated fats increase blood cholesterol levels, thus contributing to cardiovascular disease risk. The White American subjects were successful in reducing their average percentage of saturated fat intake from 13.44% to 10.15%, and the African American subjects successfully reduced their average percentage of saturated fat intake from 13.86% to 10.72%. Hu et al. completed a study in 1999 that indicated a diet that replaces saturated fat intake with polyunsaturated fat intake has been successful in lowering cardiovascular disease risk [32]. However, a review that looked at recent studies of how saturated fat intake related to cardiovascular disease indicated that there was a weak association with saturated fat intake and cardiovascular disease risk. The author instead suggested that the types of nutrients that replace the saturated fat may have more to do with the decreased risk [33]. Our study did not account for replacing saturated fat with

polyunsaturated fats or other nutrients, so we are unable to make these same conclusions.

V.4 Vitamin Intake

The National Institute of Health fact sheet states that the RDI for Vitamin E is 15 mg/day, Vitamin C is 75-90 mg/day, and Carotenoids are 700-900 µg/day [26]. The fact sheets also indicate that the national average of intake for Vitamin E is between 19.3-24.9 mg/day, for Vitamin C is 83.6 mg/day for women and 105.2 mg/day for men, and for Carotenoids are 580 µg/day for women and 649 µg/day for men [26]. In our study, we found that the vitamin intake of E, C, and Carotenoids were above the RDI and national average at the baseline. A positive aspect of our weight loss intervention was that the caloric intake decreased, the intake of vitamins increased as shown on **Figures 1 and 2**. The SlimFast shakes accounted for some of these vitamins intakes, with a single shake having around 15 mg of Vitamin E (100%), 75-90 mg Vitamin C (100%), and 245-315 µg of Carotenoids (35%) [21]. We can assume from the numbers reported in the food frequency questionnaires that the consumption of these vitamins were increased. We can also assume that the other factor for the increase of vitamin intake comes from the types of snacks and full meal the subject chose to eat, as there are certain foods that are high in the vitamins with antioxidant functions. Foods high in Vitamin E are nuts, seeds, vegetable oils, green leafy vegetables, and fortified cereals; foods rich in Vitamin C are citrus fruits, tomatoes and tomato juices, potatoes, peppers, and strawberries; foods rich in carotenoids are carrots, sweet potatoes, dark leafy greens, and tomatoes. A 2011 study showed that higher fruit content in a low calorie diet improved antioxidant status during weight loss, and that tomato and spinach specifically (high in Vitamin E and Carotenoids) prevented oxidative stress [38]. The subjects in our study attended the 6 month “Living Well Program” at the Comprehensive Weight Management Program. This program reviewed ways to maintain a healthier lifestyle, and encouraged subjects to find way to increase their fruit and vegetable intake as a way to get these antioxidant vitamins. Therefore, it

adding additional benefits for the subjects involved in the Weight Management Program.

V.5 Lipids and Lipoproteins

Elevated lipids and lipoproteins are associated with increased cardiovascular disease risk. In our study, we found HDL to be below the recommended value of 50 mg/dL before and after our study. Similarly, LDL-C followed the same trend with the LDL levels over the value of 100 mg/dL before and after our study. An increased HDL-C amount is important because HDL-C works to collect LDL-C and recycle it back to the liver, therefore preventing its oxidation. HDL-C also works to keep the endothelial lining of the artery healthy. A high amount of LDL-C would mean that more would be available to be oxidized, and therefore more plaque would be formed on the arterial wall. A study performed in 1994 demonstrated that although Vitamin C is hypothesized to increase plasma HDL-C concentration, supplementation has not been shown to have a significant increase in the plasma levels [34]. This did not correlate with the results seen in our study, since an increase in Vitamin C intake did not yield a significant increase in plasma HDL-C concentrations. Other studies have also indicated no significant association between fruit and vegetable intake that contain these antioxidant vitamins and HDL-C concentration [6]. While the vitamin intake in our study did not correlate with changes in plasma lipid concentration, a study performed in elderly cardiovascular patients indicated that increased antioxidant supplementation (specifically Vitamin E) resulted in decreased lipid oxidation [38]. However, while they received positive results, other studies have not shown this same result with Vitamin E supplementation [39]. The authors noted that that the decreased lipid oxidation could have come from an increase of whole fruits and vegetables intake in patients who were already suffering from cardiovascular disease. Our study had vitamin supplementation in the Slimfast meal replacement shakes and vitamins from whole foods in the snacks and balanced meals, but we did not directly measure what caused an increase of vitamins in our subjects.

Most large epidemiological studies show that AA have an increase in HDL when compared to WA, however while our study showed this increase in HDL levels in AA subjects, the increase was not significant either at the baseline or 6 month period [19,41]. Triglyceride levels have also showed to be lower in AA when compared to WA [19,41]. Our study only showed AA to have significantly lower triglycerides at the baseline, but not at the 6 month mark. The dietary induced weight loss program was successful in decreasing the cholesterol levels in WA subjects, bringing these levels to that of the AA subjects. The AA subjects in our group did not have a significant decrease in triglycerides, what may be related to their already lower numbers. Triglyceride levels are related to the amount of calories and fat in the diet because they store the unused calories that are not burned. Therefore, since the WA saw a significant decrease in calories, they also saw this significant decrease in triglycerides, while AA subjects did not see either. A larger population size could have increased the significance in the changes we saw between the AA and WA subjects.

V.6 Inflammatory Markers

Inflammation is considered a risk factor for cardiovascular disease, which can be caused by increased oxidation in the body. The inflammatory markers, such as c-reactive protein (CRP) and oxidized LDL also exhibited no change over the 6-month period. C-reactive protein is found in the blood in response to an increase in inflammation. The CRP binds to macrophages that attend to damaged cells, and are present in states of increased inflammation. CRP levels associated with an elevated risk of inflammation in the body and therefore cardiovascular disease are greater than 3.0 mg/dL. Based on the CRP levels at the end of the study, subjects had levels that were close to the normal levels. A 2006 study demonstrated the opposite results. The authors found an inverse relationship between vegetable intakes and CRP levels. A major difference between this study and ours was that not all the patients in their study were overweight or obese, and only some of them were characterized as having metabolic syndrome. In our study, all of the subjects were

dysglycemic and overweight/obese, therefore having 2 cardiovascular disease risk factors. These differences may contribute to findings between the study. The study comments that the correlation of CRP and vitamin intake could have only been present in the healthy subjects. They concluded that this might be related because of the assumption that they lead generally healthier lifestyles [6]. An additional study that placed subjects on a restricted calorie diet for two years (1300 kcal/day for the first year; 1500kcal/day for the second year) saw reductions of CRP in subjects after losing 15 kg of weight and 5.2 kg/m² in their BMI. This study showed the effectiveness of reducing CRP levels during a long term weight loss program [37], in contrast to our 6 month study.

Oxidation of LDL is closely associated with increased cardiovascular disease risk. Diets high in vitamin intake are typically associated with decreased LDL oxidation. Our study found that subjects did not change their oxidized LDL after the 6-month period. Studies that have found decreased levels of oxidized LDL have noted that oxidized LDL levels decrease because of very low caloric intake (580 kcal/day [34]) or significant weight loss of 13 kg or more [36]. Our subjects were over the 580 kcal/day mark and lost less than 13 kg of weight over the 6 month period. Thus, it could be that if our study was extended longer, we would have demonstrated decreased oxidized LDL, as seen in the above study. A 2011 study that focused on the specific increase of antioxidant containing fruit and vegetable intake in healthy adults saw an inverse relationship between fruit and vegetable intake and oxidized LDL levels, especially in the diets that included only fruit juices [40]. The dysglycemic state of the subjects in our study could have prevented this relationship to occur. The lack of significant change in these inflammatory markers indicate that the high antioxidant vitamin intake might not correlate to lower amounts of oxidized LDL in the blood stream. However, the lack of significant change could also be attributed to bioavailability of the antioxidants in the vitamins, or the way the vitamins are obtained (whole foods versus supplementation) [40]. This lack of significant change was also seen in studies regarding vitamin supplementation [7,14,18]. While there are varying results, there is no direct claim to one form of vitamin intake being better than the other. Even though we had a combination of

vitamin intake, we did not see a decrease in the inflammatory markers. Therefore, additional studies are warranted to examine the benefits of vitamin supplements or vitamins from whole foods and weight loss as well as different sources of these vitamins.

V.7 Limitations

Our diet induced weight loss program resulted in reduction of weight, caloric intake, and blood pressure. We also saw an increase in overall intake of Vitamins E, C, and Carotenoids among our subjects. While we saw these positive changes, we did not see a change in lipid, lipoprotein, or inflammatory marker levels measured in our study. We also did not see metabolic changes through glucose and hemoglobin A1C levels. However, there are several limitations in our study that warrant discussion. We used a food frequency questionnaire to obtain the levels of caloric, macronutrient, and vitamin intake. Since the food frequency questionnaire compiles the general food intake over the past 3-months, it relies on subjects' recall of various aspects of their diet. This limited us because of the potential challenges of remembering food intake over the past 3 months, which may not be as accurate as a 3-7 day food record or a 24-hour recall. Additional studies have also noted the potential inaccuracy with food frequency questionnaires because of their dependency on individual reporting [12].

The food frequency questionnaire alone does not provide the serum levels of the vitamins, and does not account for if the vitamin is in its most bioavailable form. In our study, we reported only the dietary intake of vitamins, therefore we could not accurately correlate increases in vitamin intake with serum levels of the vitamins. By using the serum level in conjunction with 24 hour recall and asking specific questions related to fruit and vegetable intake it could be possible that we may observe significant increases in both dietary and serum levels of Vitamin E, C, and Carotenoids. Thus allowing us to look more accurately at the relationship between antioxidant vitamins and risk for cardiovascular disease.

We observed a significant increase of reported dietary intake of Vitamin E, C, and Carotenoids from the food frequency questionnaires. These results can be

confounded because of the high caloric intake of the subjects at the beginning of the study, therefore yielding an increased intake of the vitamins in general.

We chose SlimFast 3-2-1 plan because it was easy for participants to follow, and we hoped it would encourage subjects to continue to lose weight once the study ended. A better dietary intervention could be to have one-on-one consultations with a dietician to develop a more individualized meal plan that contained foods the subjects enjoyed, therefore potentially increasing their weight loss and healthy food intake. Finally, we did not measure physical activity level in the subjects. It could be possible that the combination of decreased caloric restriction and physical activity would have resulted in increased weight loss and decreased BMI and Waist Circumference in our subjects, specifically the AA subjects. A long term weight loss study performed with AA and WA women found that AA had higher odds of achieving weight loss by decreasing calories and focusing on their fast food and soft drink consumption. This study also noted that physical activity was a large factor in achieving long-term weight loss rather than regaining the weight after a 5-year period [45]. In addition, we believe that if our study was expanded to 1 year or more, we would have seen a more remarkable weight loss in our subjects.

Finally, we did not measure physical activity level in the subjects, although the importance of physical activity was discussed as a part of the Comprehensive Weight Management program we did not accurately perform any measure of physical activity. It is possible that better results would have been achieved if this was incorporated at part of the program. In the Diabetes Prevention Program, we saw positive results of physical activity and weight loss when we looked at the Diabetes Prevention Program [42,43], and the Look Ahead Trial [29]. Previous studies have also shown that physical activity greatly impacts weight loss, cholesterol concentrations, and inflammatory markers [38]. Including a physical activity factor into our study could have also allowed us to see more significant changes in weight loss, body composition, and inflammatory markers, thus reducing multiple cardiovascular disease risk factors.

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